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EFFICACY OF *TRICHODERMA* SPP, NEEM PRODUCTS AND CARBENDAZIM AGAINST FUSARIAL WILT OF TOMATO (*LYCOPERSICON ESCULENTUM* L.) IN POT CONDITIONS

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ABSTRACT

The study was conducted to investigate efficacy of *Trichoderma* spp, Neem products and carbendazim against *Fusarium oxysporium* f.sp. *lycopersici* wilt of tomato and growth parameter of tomato plants in pot condition. A total of six treatments, replicated four times were taken up in randomized block design. The pots were inoculated with *Fusarium oxysporium* f. sp. *lycopersici* culture 10g/kg of sterilized soil (for making of infected soil). *Trichoderma harzianum* 4g/kg of seed+5g/kg of soil as soil application, *Trichoderma viride* 4g/kg of seed+5g/kg of soil as soil application, Neem cake powder 7g/pot as soil application, Neem leaves extract 1:2 concentration seed treatment+10% concentration. Neem leaves extract 3liters/m^2 seedbed application and carbendazim 2g/kg of seed+1.5g/kg of soil as soil application. One month old tomato seedlings were transplanted 4 seedlings per pots the data were recorded at 30, 45, 60 and 75 days after transplanting. All treatments were significantly reduced disease severity of *Fusarium oxysporium* f.sp. *lycopersici* as compared to control. The significantly reduced per cent disease sevirity was recorded in treatment carbendazim (16.9%) followed by *Trichoderma harzianum* (18.10%), *Trichoderma viride (*19.50%), Neem leaves extract (20.70%) and Neem cake powder (22.09%) including with control pots (26.01%). All treatments were significantly increased plant height, root length, fresh and dry shoot-root weight and yield per plant.

KEYWORDS: Carbendazim, *Fusarium oxysporum* f.sp. *lycopersici*, Neem Products, Tomato, *Trichoderma* spp

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) belongs to family solanaceae. Tomatoes are the world's largest vegetable crop and known as protective food both because of its special nutritive value. Tomato is one of the most important vegetable crops cultivated for its fleshy fruits, it is considered as important commercial and dietary vegetable crop. Its fruit is rich in vitamins and is therefore used in salads, cooked as a vegetable or made into tomato paste, ketch-up, sauce, chutney, soup, paste, puree etc. Tomato is a rich source of minerals, vitamins and organic acid, essential amino acids, dietary fibers, and Vitamin A and C, it also contains minerals like iron, phosphorus, and tomato also contains lycopene and Beta-carotene pigments. It also contains minerals like iron and phosphorus. It plays an important role in maintaining the human health. Being rich source of lycopene, tomato is used in the treatment of cancer, especially the prostate cancer [5]. One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production. Fungal phytopathogens are cause of many plant diseases and much loss of crop yields, especially in tropical and subtropical regions. *Fusarium oxysporum* is major soil-borne fungal pathogen of both greenhouse and field grown tomatoes in the warm vegetable growing areas of the world. Fusarial wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici.*

Based on the Food and Agriculture Organization report, tomato fruits for fresh market and processing were produced worldwide on approximately 4 million hectares with yearly worldwide fruit production being 107.9 million tonnes [3]**.** One of the most important diseases of tomato in the warm regions of the world is Mill) is one of the most important solanaceous vegetable crops grown worldwide. The disease is characterized by wilting, yellowing of leaves and minimal or absent of crop yield. It is a highly destructive pathogen, both in the glasshouses and in the fields causing 10 to 50% yield loss in many tomato production areas.

This study was undertaken to evaluate the efficacy of *Trichoderma* spp, Neem products and carbendazim against Fusarial wilt of tomato (*Lycopersicon esculentum* L) when used as a seed treatment and soil application in pots conditions for the management of *Fusarium oxysporum f.sp. lycopersici*. [10] studied some chemical compounds have been successfully used to control soil borne plant pathogens. Although in many cases, these pesticides appear to be the most economical and efficient means of controlling plant pathogens. Toxicological environmental and sociological concerns have led to drastic reduction in the availability of efficient commercial compounds, and also the fungicides may lead to the appearance of new resistant strains of pathogens. [17] reported that treatment of infested banana fruit with aqueous leaf extract of *A. indica* gave good control of *F. oxysporum* disease development with minimum percentage loss in fruit weight and was showed to be among the most effective medicinal plants used. [4] reported that aqueous neem leaf extract inhibited mycelial growth and spore germination of *Helminthosporium oryzae* and *pyricularia oryzae* responsible for blast and brown spot of rice plant respectively. [18] showed the fungicidal properties of aqueous leaf extracts of *Azadirachta indica* against *Alternaria alternate* from pear fruits with 85 % control of fruit rot in vivo.

MATERIALS AND METHODS

The experiment was conducted under pots condition in the Department of Plant protection, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad (India) during the year 2013. The earthen pots were disinfected with 4% formalin solution. The formalin solution was mixed with the soil and that was covered with polythene bag for 48 hour. After wards when the trace of formalin smell has gone the soil was worked up thoroughly dried and used in to pots.

Isolation and Purification of *Fusarium oxysporum* f. sp. *lycopersici*

The infected portion of tomato (stem and root) was cut into 2-3 mm. small pieces. These were surface sterilized with 0.1 percent mercuric chloride solution for one minute then rinsed in sterilized distilled water, blotted and plated on czapekdox agar medium (sodium nitrate 3g, potassium chloride 0.5g , Di-potassium hydrogen 1g , sucrose 30g, Magnesium sulphate 0.01g, Agar 15g, and water1 liter). On 99 mm Petri-plates three pieces per plate were arranged. These plates were incubated in 20±2 ^{0}C for 5 days to recover pathogen. The colonies of tomato wilt fungus along with some other colonies i.e. air-borne fungi were recovered. This Fusarial wilt fungus was purified by single spore method [1].

Soil Infestation

Soil was infested with the mass culture of *Fusarium oxysporum* f.sp. *lycopersici*, which was multiplied with sorghum grains the soil was inoculated with 10g *Fusarium* mass culture per kilogram of soil. After that the soil was potted in earthen pots.

Pathogenicity Test

The pathogen of F*usarium oxysporum lycopersici* was isolated from infected tomato root and stem portion and purified after identification. Isolate was test on15days old tomato seedling at the three leaf stage. Their roots were dipped in to a conidial suspension (10^6 spores/ml) for 10 minutes then the seedling were transplanted into sterilize soils in pots and

kept in green house after symptoms appeared was same to *fusarium* symptoms and the pathogen was re-isolated from infected portion of infected tomato seedling of tomatoes and again identified in microscopic examination in laboratory.

Details of Treatment

The present study was undertaken to evaluate the efficacy of *Trichoderma* spp, neem products and carbendazim against Fusarial wilt of tomato (*Lycopersicon esculentum* L) caused by *Fusarium oxysporum* f.sp. *lycopersici.* The seed was treated with *Trichoderma harzianum* @4g/kg of seed+ 5g/kg of soil as soil application and *Trichoderma viride* @ 4g/kg of seed as seed treatment+5g/kg of soil as soil application, Neem kernel cake @ 7g/per pot, seed treated with Neem leaf extract @1:2 as seed treatment+ seedbed treatment with 10% Neem leaf extract @ 3 liter/m^2 seedbed and seed treatment with carbendazim @2g/kg of seed as seed treatment+soil application @1.5g/kg of soil as soil application. Control pots without treatment which inoculated with 12 days old culture of *Fusarium oxysporum* f.sp. *lycopersici.* @ 10g/kg of soil and one month old tomato seedling were transplanted 4 seedling per pot and kept under open sky and the data were recorded at 30, 45,60, and 75 days after transplanting.

RESULTS AND DISCUSSIONS

Results of interactive effect of *Trichoderma* spp, neem products and carbendazim against Fusarial wilt of tomato (*Lycopersicon esculentum* L.) caused by *Fusarium oxysporum* f.sp. *lycopersici* on different parameters *viz*. plant height, disease severity, root length, fresh and dry shoot, root weight and yield/plant are presented in Table: 1.

Plant Height (cm)

After thirty, forty five, sixty and seventy five days of transplanting data observed that plant height (cm) was significantly increased in Neem cake powder (27.08, 42.08, 55.17 and 65.00 cm) as compared with *Trichoderma harzianum* (25.33, 40.33**,** 53.17 and 63.18cm), *Trichoderma viride* **(**23.08, 37.25, 50.08, 60.00cm), carbendazim (21.08, 35.83**,** 47.75 and 57.83cm), Neem leaves extract (22.00**,** 30.75**,** 42.92 and 52.50cm) including control (16.67, 24.83, 33.42 and 43.25 cm).

Disease Severity (%)

The results revealed that the carbendazim was the best treatment (4.84%) with lowest disease severity followed by *Trichoderma viride* (6.7%), *Trichoderma harzianum* (7.86%), Neem leaves extract (8.00%) and Neem cake powder (9.51%). Whereas, maximum disease severity was recorded in control pot. At 45 days after transplanting the best treatment was carbendazim which had lowest (8.36%) disease severity followed by *Trichoderma harzianum* with (9.53%), *Trichoderma viride* (10.36 %), neem leaves extract (11.00%), Neem cake powder (12.25%). Sixty days after transplanting the best treatment was carbendazim which had lowest disease severity (12.27%) followed by *Trichoderma harzianum* with (14.24%), *Trichoderma viride* (15.56%), Neem leaves extract (16.62), Neem cake powder (17.08%). At 75 days after transplanting the best treatment was carbendazim which had lowest disease severity (16.9%),followed by *Trichoderma harzianum* (18.10), *Trichoderma viride* 19.50%, Neem leaves extract (20.70%), neem cake powder (22.09). during 30,45,60,75 days after transplanting the control pots had 10%,15%,20.30%,and 26.01% disease severity. All treatments were significantly reduced the disease severity compared to control. Among the all treatments the most effective treatment was carbendazim and the least effective was Neem cake powder.

Root Length at 90 Days after Transplanting

Ninety days after transplanting the tomatoes plant uprooted and the root was measured the maximum root length (19.00 cm) was recorded in T_1 *Trichoderma harzianum*, followed by (18.08 cm), T_2 *Trichoderma viride*,

(17.00cm), Neem cake powder, (14.00 cm), T_3 carbendazim, (13.50 cm) and T_5 Neem leaves extract (13.5 cm) including with control (10.2 cm). However, the treatments (T_2, T_4) and (T_3, T_5) were non significant among themselves.

Fresh Shoot Weight at 90 Days after Transplanting

Tomato plant fresh shoots were weighted on an electronic balance and the data was recorded, the maximum fresh shoot weight (40.00g) was recorded in T_1 *Trichoderma harzianum* followed by T_2 *Trichoderma viride* (38.50 g), T_5 Neem leaves extract (35.25g), T_4 Neem cake powder (32.50g) and T_3 carbendazim (31.0g) including with control (29.0g). Whereas, the treatments (T_2, T_5), (T_5, T_4) and (T_4, T_3 and T_0) were found non-significant among themselves.

Dry Shoot Weight at 90 Days after Transplanting

Plants shoot were dried in an oven at 70^0C until constant weight. Results revealed that maximum dry soot weight was recorded in T_1 *Trichoderma harzianum* (9.00g) as compared with T_2 *Trichoderma viride* (7.50 g), T_4 Neem cake powder (6.25g), T_3 carbendazim (5.00gm) and T_5 Neem leaves extract (4.30g) including with control (3.5g). Whereas, the treatments (T_2, T_4), (T_4, T_3) and (T_3, T_5 and T_0) were found non-significant among themselves.

Fresh Root Weight

The maximum fresh root weight was recorded in T_1 *Trichoderma harzianum* (10.00g) followed by T_2 *Trichoderma viride* (8.50 g), T_4 neem cake powder (6.50g), T_3 carbendazim (5.50g) and T_5 Neem leaves extract (4.25g). Whereas, the treatments (T_4, T_3) and (T_3, T_5) were found non-significant among themselves. The minimum fresh roots weight was recorded in T_0 control pots.

Dry Root Weight (gm) at 90 Days after Transplanting

Results showed that maximum dry root weight was recorded in T_1 *Trichoderma harzianum* (3.50g) followed by T_2 *Trichoderma viride* (2.50g), T_4 Neem cake powder (1.94g), T_3 carbendazim (1.50g) and T_5 Neem leaves extract (1.00g) including with control (0.5). The minimum dry root weight was recorded in T_0 control pot.

Yield per Plant

The maximum yield per plant was recorded in treatment T_1 *Trichoderma harzianum* (508.00g) followed by T_4 Neem cake powder (475.00g), T_2 *Trichoderma viride* (441.00 gm), T_3 carbendazim (416.00 g) and T_5 Neem leaves extract (400g). Whereas, the minimum yield was recorded T_0 control pots.

Table 1: Effect of T*richoderma* spp, Neem Products and Carbendazim on Different Parameters of Tomato Plants

Treatments	Plant Height (cm.)				Diseases Severity (%)				Root Length (cm.)	Fresh Shoot Weight (gm)	Dry Shoot Weight (gm)	Fresh Root Weight (gm)	Dry Root Weight (gm)	Yield Per Plant (gm)
	30 DAT	45 DAT	60 DAT	75 DAT	30 DAT	45 DAT	60 DAT	75 DAT						
T. harzianum	25.33	40.33	53.17	63.17	7.86	9.53	14.24	18.10	19.00	40.00	9.00	10.00	3.50	508.00
T. viride	23.08	37.25	50.08	60.00	6.70	10.36	15.56	19.50	18.00	38.50	7.50	8.50	2.50	441.00
Carbendazim	21.08	35.83	47.75	57.83	4.84	8.36	12.27	16.9	14.00	31.00	5.0	5.50	1.50	416.00
Neemcake powder	27.08	42.08	55.17	65.00	9.51	12.25	17.08	22.09	17.00	32.50	6.25	6.50	1.94	475.00
Neem leaves extract	22.00	30.75	42.92	52.50	8.00	11.00	16.62	20.70	13.50	35.25	4.30	4.25	1.00	400.00
Control	18.67	25.83	34.42	43.25	10.92	15.00	20.30	26.01	10.25	29.00	3.50	3.25	0.50	320.00
F- test	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Ed. (±)	1.34	2.26	3.71	2.99	0.24	0.22	0.25	0.48	0.92	2.01	0.96	0.90	0.26	25.71
C. D.(P = 0.05)	2.85	4.80	7.87	6.34	0.51	0.47	0.53	1.02	1.95	4.27	2.04	1.91	0.56	54.52

DISCUSSIONS

Similar finding reported by **[6]** studied that treatment of *T. harzianum, T. viride and Pseudomonas* on tomato plant gave maximum control of wilt disease with *T. harzianum* (44.4%) treated plants as compared to FOL inoculated plants and all treatments were able to boost plant growth and provide significant reductions in disease levels. The maximum wilt control observed in tomato plants treated with *Trichoderma harzianum*. **[9]** reported that the result of the experiment revealed that seed treatment with soil application of *Trichoderma* spp were found more effective in enhancing the growth and suppress the wilt disease severity and also seed treatment plus soil application of *Trichoderma virens* was most effective and significantly improved the plant growth. **[8]** reported that in case of Fusarial wilt of tomato at 25 days after sowing the significantly highest germination percentage and lowest percentage of fusarial wilt disease of tomato were recorded in seed treatment with neem leaves extract. The significant highest performance of growth characters of tomato seedling were observed where seeds were sown after treated with Neem leaves extracts. **[11]** reported that tomato plants grown in soil amended in different rates of NKCP differed (p=0.01) significantly in their performance. Disease severity based on the wilt index (0.53-2.28) and length of discolored vascular tissues (7.4cm-25.62cm) differed significantly (p=0.05) among the treatments. **[7]** reported the mycoparasitism inhibitory effects of five *Trichoderma* species (*T. harzianum, T. koningi, T. longiconis*, *T. hamatum and T. viride*) on the growth of the causal agent of tomato Fusarial wilt (*Fusarium oxysporum* f.sp. *lycopersici*) were investigated by dual culture in laboratory condition. In this step, the maximum and minimum inhibitory effect was caused by *T. harzianum* and *T. viride*. In the greenhouse, the comparison of the efficacy of disease decrease was carried out between soil and seed treatments affected by *T. harzianum* spores. Results showed that seed treatment did not cause disease decrease but soil treatment caused disease decrease by 92%. [14] reported that neem leaf extract was effective in reducing blight incidence with increased yield of tomato infected by *Alternaria solani.* **[13]** reported that all critical appraisal of treatments revealed that there is an effective control of chili with seedling dip in carbendazim (0.1%) and drenching of plants at flowering stage either with carbendazim (0.1%) or mancozeb (0.25%). **[12]** reported that integrated treatments with neem products and *B. subtilis* gave maximum disease control (65.33%) and maximum increased in height of tomato**. [15]** reported that different fungicides viz., Carbendazim, Dithane M-45. Thiovit and Thiophanate-methyl were significantly reduced the growth of *F.oxysporum* f.sp. *Vasinfectum* compared to control. The most effective fungicides were found to be Carbendazim followed by Thiophanate-methyl. [16**]** reported *Trichoderma* species are commonly used as biological control agents against phytopathogenic fungi and increase shoot height, shoot diameter, shoot fresh and dry weight and root fresh and dry weight in tomato seedlings were interestingly ($p \leq 0.05$) increased when sown in *Trichoderma* sp. **[1]** studied the growth promoting ability of *T. koningii* and a white sterile fungus (which did not fructify) on tomato plants grown in soil inoculated with wilt pathogen *F. oxysporum* f. sp. *lycopersici.* He reported that antagonistic rhizosphere PGPF suppressed the deleterious soil microbes by competing at the active sites, reduced the intensity of disease development and subsequently, stimulated the growth and yield of tomato plants.

SUMMARY AND CONCLUSIONS

The present study concludes that effect of *Trichoderma* spp, Neem products and Carbendazim against *Fusaraium oxysporium* f.sp. *lycopersici* causative agent of fusarial wilt of tomato. From the critical analysis of the present findings it was concluded that among all the treatments, carbendazim was significantly reduced disease severity as compared to other treatments. In the plant height was significantly increased in treatment Neem cake powder and least effective was neem leaves extract. In the root length shoot fresh and dry weight root fresh and dry weight, yield per plant most effective

treatment was *Trichoderma harzianum.* These can be further tried and evaluated against various plant diseases. But it still needs more investigation to be conducted in this regard for proper recommendations.

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APPENDICES

Figure1: Pure Culture of *Fusarium oxysporum* f.sp. *lycopersici*

Figure 2: Micro and Macro Conidia of *Fusarium oxysporum* f.sp. *lycopersici*

Figure 3: Yield of Tomato Plant in Pots